

**Influence of pyroligneous extract on water quality and productive performance of *Penaeus vannamei*****Influência do extrato pirolenhoso na qualidade de água e no desempenho produtivo de *Penaeus vannamei***

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**Thales da Silva Moreira**

Mestre pela Universidade Federal do Ceará

Programa de Pós-Graduação em Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil

E-mail: thalesparakas@hotmail.com

**Ana Luzia Assunção Cláudio de Araújo**

Mestre pela Universidade Federal do Ceará

Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil

E-mail: analuzia\_aca@hotmail.com

**Thiago Bastos Bezerra de Menezes**

Mestre pela Universidade Federal do Ceará

Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil

E-mail: thiagobezerra@gmail.com

**Kamar Porto do Nascimento Filho**

Mestre pela Universidade Federal do Ceará

Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil

E-mail: kamarfilho\_12@hotmail.com

**Rebeca Larangeira de Lima**

Mestre pela Universidade Federal do Ceará

Programa de Pós-Graduação em Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil

E-mail: rebecalarangeira@gmail.com

**Rommel Rocha de Sousa**

Doutor pela Universidade Federal do Ceará  
 Programa de Pós-Graduação em Engenharia de Pesca, Campus do Pici, Universidade  
 Federal do Ceará, 60.455-760, Fortaleza, Ceará, Brasil  
 E-mail: rommelpesca@gmail.com

**João Felipe Nogueira Matias**

Doutor pela Universidade Federal do Ceará  
 Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará,  
 60.455-760, Fortaleza, Ceará, Brasil  
 E-mail: jfn.matias@gmail.com

**José Renato de Oliveira César**

Doutor pela University of Hawaii  
 Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará,  
 60.455-760, Fortaleza, Ceará, Brasil  
 E-mail: renatocesarufc@gmail.com

**Elenise Gonçalves de Oliveira**

Doutora pela Universidade Estadual de São Paulo  
 Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará,  
 60.455-760, Fortaleza, Ceará, Brasil  
 E-mail: elenisego@yahoo.com.br

**Francisco Hiran Farias Costa**

Doutor pela Universidade Federal do Ceará  
 Departamento de Engenharia de Pesca, Campus do Pici, Universidade Federal do Ceará,  
 60.455-760, Fortaleza, Ceará, Brasil  
 E-mail: hiranfcosta@gmail.com

**ABSTRACT**

Water is the main input used in shrimp farming, and the good quality of this natural resource is not only essential for the farmed animals' welfare, but also for a good profitability at the end of the farming process in aquaculture species. Pyroligneous extract (PE) is a yellow to reddish-brown liquid obtained from the condensation of gases produced by partial wood combustion. This compound has been used for several purposes in different areas, such as in the fertilization of various vegetable crops and in soil conditioning. The objective of the present study was to evaluate the possible benefits of the application of pyroligneous extract on water quality and its consequences on the production performance of *Penaeus vannamei*. Six 1.0 ha ponds were used, 3 of which were used for the application of pyroligneous extract (WPE) and the other 3 without the extract (wPE) for comparison purposes. The extract was diluted in water and applied directly to the dry soil and after a few hours of reaction the ponds were filled with water and prepared for cultivation. There was a significant difference ( $p < 0.05$ ) between the ponds (WPE) and (wPE) in the following water quality parameters: dissolved oxygen, pH, total alkalinity, total hardness, magnesium and silica. However, the values found

were within the recommended range for shrimp farming. No significant differences ( $p>0.05$ ) were found in the parameters analyzed in relation to production performance.

**Keywords:** Pyroligneous extract; Plankton; Water quality.

## RESUMO

A água é o principal insumo utilizado na carcinicultura e a boa qualidade desse recurso natural no cultivo de camarões é de essencial importância para o bem estar dos animais cultivados e para uma boa lucratividade ao final do cultivo das espécies de interesse aquícola. O extrato pirolenhoso (EP) é um líquido de cor amarelo à marrom avermelhado obtido da condensação dos gases produzidos da combustão parcial de madeira. Esse composto tem sido usado para diversas finalidades em diferentes áreas, como na fertilização de várias culturas vegetais e no condicionamento do solo. O objetivo do presente trabalho foi avaliar os possíveis benefícios da aplicação do extrato pirolenhoso na qualidade de água e as suas consequências no desempenho produtivo de *Penaeus vannamei*. Foram utilizados seis viveiros de 1,0 ha, sendo 3 usados para a aplicação do extrato pirolenhoso (CEP) e os outros 3 sem o extrato (SEP) para fins de comparação. O extrato foi diluído na água e aplicado diretamente no solo seco e após algumas horas de reação os viveiros foram abastecidos com água e preparados para o cultivo. Houve diferença significativa ( $p<0,05$ ) entre os viveiros (CEP) e (SEP) nos seguintes parâmetros de qualidade de água: oxigênio dissolvido, pH, alcalinidade total, dureza total, magnésio e sílica, entretanto os valores encontrados estavam dentro da faixa recomendável para cultivo de camarões. Em relação ao desempenho produtivo não foram constatadas diferenças significativas ( $p>0,05$ ) nos parâmetros analisados.

**Palavras-chave:** Extrato pirolenhoso; Plâncton; Qualidade de água.

## 1 INTRODUCTION

Many industrial processes use the burning of wood to generate energy to manufacture several products. One of the obstacles faced in this practice is the generation of gases from wood combustion that contribute to air pollution. The carbonization of wood, besides generating energy, also generates products such as charcoal and by-products derived from smoke, and the use of the compounds synthesized from the gases generated by wood burning can reduce the environmental impacts caused by this activity, contributing to the reduction of global warming (PORTO *et al.*, 2007; SOUZA *et al.*, 2018).

The recovery and subsequent condensation of volatile gases formed in charcoal production sites is an old technique for obtaining pyroligneous extract. There are reports of the use of this compound for millennia in China and India. However, the first researches with the pyroligneous extract were only performed in 1874 in Japan (MIYASAKA *et al.*, 1999;

CAMPOS, 2007). Currently, the technologies used in the carbonization of wood in the production of charcoal generate large masses of by-products, and around 70% are gases and tar, which have high energy value and are not used (JESUS, 2016).

Pyroligneous extract is a yellow to reddish-brown liquid, depending on the refining process, resulting from the condensation of the smoke produced in the combustion of wood from different plant species such as eucalyptus, bamboo and pine in charcoal production. This extract is also known as pyroligneous acid, a term used in scientific studies, and is also known as wood vinegar, pyroligneous liquor and liquid smoke (ALVES, 2006; SOUZA-SILVA *et al.*, 2006).

According to Miyasaka *et al.* (1999), the use of pyroligneous extract in agriculture must go through a purification process and cannot be used in the raw form, as it has a certain amount of tar that is a toxic compound for both plants and people who will handle the product, so it is necessary that the pyroligneous extract remains at rest for a period greater than 100 days for the decanting of the tar to occur. This rest promotes a separation of the condensate in three phases: oils with low density, the pyroligneous extract and the tar in this sequence from top to bottom.

Research conducted with farmers in Japan using pyroligneous extract found its action as repellent for some species of pests in crops, such as birds, bats and rodents, its benefit in preventing disease in crops, action on animal feces in order to eliminate the strong odor (ENCARNAÇÃO, 2001), improving the vegetative development of some crops, organic fertilization, soil conditioning and to facilitate the rooting of certain plant crops (SCHNITZER *et al.*, 2015).

One of the key factors for increasing crop productivity and improving product quality is fertilization (PACHECO *et al.*, 2008). The increase in food production is directly related to innovation in the use of fertilizers in agricultural systems, so without the use of this resource, current production levels would not reach this level. Correct fertilization management directly influences the reactions between soil and fertilizer by effectively making nutrients available to plants (FIORIN *et al.*, 2016). Several types of fertilizers with different chemical compositions, nutrients, efficiency and granulometry are offered on the market (HANSEL *et al.*, 2014).

In aquaculture, the practice of pond fertilization is important to increase the availability of nutrients for phytoplankton development and thus increase primary production in pond water (BOYD; TUCKER, 1998). Natural food production is essential to improve the

digestibility of cultivated organisms, especially in the larval stages, and consequently to promote optimal zootechnical performance (PEDREIRA *et al.*, 2008). In fish farming, fertilization performed under controlled conditions is a very important management practice, as it promotes an increase in the culture potential (MACEDO; SIPAÚBA-TAVARES, 2010).

Several experiments were performed with different types of organic fertilizers in fish production, mainly with animal manure, such as poultry (RABIA *et al.*, 2015), cattle (RAPATSA; MOYO, 2013; NAIR *et al.*, 2014), and swine (BWALA; OMOREGIE, 2009). This type of organic fertilization, if not properly performed, can lead to health problems resulting from contamination by bacteria belonging to the coliform group, and an alternative for the control of these microorganisms is the use of pyroligneous extract (CHIAMENTI *et al.*, 2016).

The benefits of the application of pyroligneous extract are observed by several researchers, mainly in agriculture (ENCARNAÇÃO, 2001; CAMPOS, 2007). In agriculture, this product is used as organic fertilizer in different crops such as rice (TSUZUKI *et al.*, 2000), lemon (ZANETTI *et al.*, 2004), pine (PORTO *et al.*, 2007), lettuce (ROEL *et al.*, 2007). However, there is no record of its use in aquaculture. Therefore, the present study aimed to evaluate the possible benefits of the application of pyroligneous extract in water quality, including the planktonic community, and its consequences on the productive performance in a *Penaeus vannamei* culture.

## **2 MATERIALS AND METHODS**

The study was conducted at Monólitos Aquacultura Ltda, a company located in the city of Banabuiú (Ceará, Brazil). The property has 15.15 ha of water mirror distributed in 17 ponds, using a water recirculation system and a sedimentation basin of 1.83 ha to where the effluent from the harvest is thrown and then, after treatment, is re-used.

The property does not use the traditional farming system with commercial feed, instead it uses the concept known as aquamimicry, which is based on the application of a carbon source submitted to the fermentation process (rice bran and/or soybean) in the ponds in order to stimulate the development of zooplankton, simulating the conditions of a natural aquatic ecosystem (ROMANO, 2017). The shrimps were fed three times a day (08:00, 13:00 and 16:00h) with fermented soybean meal, distributed in 60 trays/ha, following the aquamimicry

protocol. The average salinity in the water of the cultivation area was 1 ppt. The average density used was around 40 shrimp/m<sup>2</sup>, with the use of 2HP paddle aerators.

For the application of the pyroligneous extract, dilution of 5 L of the product (Agro Pirolenhoso®, Doogneem Ltda, Jaguariúna, São Paulo) was performed in 15 L of water, using the dosage suggested by the manufacturer for most vegetable crops. With the help of a manual sprayer, 20 L of the solution were applied in three of the six ponds used in the experiment. The extract was applied directly to the soil in the three ponds subsequent to all routine soil treatment after harvesting. The pyroligneous extract was applied at 09:00h and remained in the soil until 17:00h when the water supply stage began. After 10 days of preparation, the ponds received the post-larvae of *Penaeus vannamei* (400,000 PL per pond), with initial average weight of 0.02 g, from CELM Aquicultura S.A. (Aracati, Ceará).

During the study period, daily monitoring of the dissolved oxygen level and temperature was performed with a multiparameter meter AK87. For the other water quality parameters (pH, salinity, total alkalinity, total hardness, calcium, magnesium, nitrate, nitrite, total ammoniacal nitrogen, phosphate, silica, apparent color and chlorophyll), three measurements were made during the experiment. Water samples were collected and analyzed at the Laboratory of Marine and Applied Geology, belonging to the Department of Geology, Science Center, Federal University of Ceará, (Fortaleza, Ceará), following the methodologies described by Aminot and Kérouel (2004) and APHA (2012).

The shrimps were sampled weekly to assess growth in weight. To achieve this, 200 shrimps in each pond were caught with a bottle at different points of the pond and weighed. After each sampling, the amount of fermented soybean supplied was adjusted to the average weight and biomass of each tank. At the end of the 99 days of farming, the shrimps were caught and the final survival (%), final weight (g), daily weight gain (average final weight - initial average weight/farming days, g/day), absolute growth rate (AGR, g/shrimp/week), specific growth rate ( $SGR = (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days} \times 100, \text{ \%/day}$ ), productivity (net biomass/pond area, kg/ha), and feed conversion ratio (feed consumption/net biomass) for each pond and treatment were calculated.

Plankton collection was performed at the beginning and end of the farming process, around 10:00h in eight different points of the farm, one collection point in each of the six ponds used in the experiment, sampling was performed near the drainage system and in two more points in the supply channel.

To perform the qualitative analysis, 200 mL were collected from each sample, concentrated with the aid of a plankton net of 20  $\mu\text{m}$  and then bottled and fixed with buffered formalin with sodium tetraborate. Identification was performed through bright-field optical microscopy with a binocular microscope, using the following classification keys: Anagnostidis; Komárek (1986, 1988, 1998), Bourrelly (1985), Bicudo; Menezes (2006).

For the quantitative analysis of the phytoplankton samples, 1,000 mL were collected from each sample in an amber glass bottle, fixed with acetic lugolin the proportion of 5 mL/L. For zooplankton sampling, a volume of 60 L of sample was filtered in a plankton net with 60  $\mu\text{m}$ , collecting the material retained in the collecting cup at the end of the filtering process and later recording the final volume. The phytoplankton count was performed by inverted microscopy with a Sedgewick-Rafter chamber utilizing the sediment. Field ranges were used according to the Poisson distribution with  $95 \pm 20\%$  confidence interval, after the concentration of the samples by sedimentation in a 1,000 mL specimen for 24 hours. The zooplankton was quantified by the scanning methodology using the Sedgewick-Rafter chamber with inverted microscope.

The quantity and quality of the planktonic community during the farming days were compared among the different collection points in order to observe possible changes among the analyzed groups. All the analyses were performed in the laboratory Ambiental Análises e Consultoria LTDA (Fortaleza, Ceará).

The water samples for the analysis of *Vibrio* spp. were collected in the initial and final stages of the farming process, at three different points in each of the ponds used in the experiment, with one point being near the water supply system, another in the water drainage system and the third point being near the central area of the ponds, and at three more points in the supply channel. For each sample, 21 sterile 200 mL flasks were used, three flasks for each of the six ponds used in the experiment and three more for the supply channel. The water samples were collected early in the morning (8:00h), and the bottles were stored in an isothermal box with ice and transported to BIOTRENDS Soluções Biotecnológicas LTDA laboratory (Fortaleza, Ceará). The analysis of total *Vibrio* spp. was performed by the spread plate counting technique using the specific medium thiosulfate-citrate-bile salts sucrose agar (TCBS-agar). In this procedure, the liquid samples were serially diluted in 2.5% (m/v) NaCl sterile solutions. Subsequently, aliquots of the raw sample and their respective dilutions were inoculated on the surface of the culture medium and uniformly distributed to count bacterial colonies. Each analysis was performed using three repetitions. The plates were incubated at



30°C for 24 hours. The colonies were then counted, and the results expressed in Colony Forming Units (CFU) per mL.

All the data are presented in mean  $\pm$  standard deviation, and to verify significant differences between the means of the two groups analyzed, the data were submitted to Student's t test, with the level of significance  $p < 0.05$ . PAST3.20 and Excel 2010 software were used to perform the tests.

### 3 RESULTS

The physical-chemical parameters of water quality are presented in Table 1. The production performance parameters of shrimps did not show any significant difference ( $p > 0.05$ ) between ponds with or without the use of pyroligneous extract (Table 2).

In the present study, 49 genera of phytoplankton were found, with a greater predominance of cyanophytes and chlorophytes in all collection points, ponds with application of the pyroligneous extract (WPE) and without the extract (wPE) and supply channel (Table 3).

In general, twelve different representatives in the zooplankton community were found in the samples collected at different points of the farm, with the Rotifers group having the highest number of taxa with six specimens, followed by the Crustacean and Protozoan groups, with three representatives each (Table 4). In the points collected in the supply channel, a greater frequency of individuals from the Crustacean group was observed, while in the ponds with application of the pyroligneous extract (WPE), there was a greater predominance of the organisms of the Rotifer group and in the ponds (wPE) there was an equivalence between the Rotifer and Crustacean groups.

Regarding the microbiological analyses for *Vibrio* sp., the presence of this group of bacteria was not detected in any of the water samples collected in the ponds with and without pyroligneous extract, as well as in the samples collected in the supply channel, both at the beginning and at the end of the experiment.

### 4 DISCUSSION

Among the water quality parameters, the values of dissolved oxygen, pH, total alkalinity, total hardness, magnesium and silica showed significant difference between the



treatments ( $p < 0.05$ ), but the values found were within the optimal range for cultivation (BOYD; TUCKER 1998; SÁ, 2012). However, there was no significant difference ( $p > 0.05$ ) between treatments for salinity, calcium, turbidity and chlorophyll  $\alpha$ . The values of nitrate, nitrite and phosphate in all the sampled ponds and total ammoniacal nitrogen (TAN) in the ponds with application of the pyroligneous extract showed concentration levels below the detection limit of the laboratory analyses. These parameters are within the acceptance standards for brackish waters class 1 according to Resolution N° 357/2005 of CONAMA, except for the mean value of TAN in the ponds without application of the extract, above 0.4 mg/L (CONAMA, 2005; QUEIROZ; SILVEIRA, 2006). The alkalinity of the ponds water presented values between 60 and 120 mg/L of Eq.  $\text{CaCO}_3$  recommended for shrimp farming (SÁ, 2012), while for total hardness and magnesium the results found were satisfactory and above 150 mg/L of Eq.  $\text{CaCO}_3$  and between 3 and 64 mg/L  $\text{Mg}^{2+}$  for the respective parameters (VAN WYK; SCARPA, 1999; BOYD et al., 2002).

All values obtained during the cultivation period are similar to those observed for shrimp farming in Brazil (NUNES, 2001; 2011; VILANI, 2011; MARTINS *et al.*, 2016). However, even though there were no significant differences between the zootechnical data obtained for shrimps, the results were better in ponds with application of the pyroligneous extract.

In the present study, the phytoplankton groups observed with higher predominance are similar to those described by McIntosh *et al.* (2006) and Brito *et al.* (2009; 2016) in shrimp farming in waters with low salinity. The most abundant groups were cyanophytes and chlorophytes with nineteen genera each, followed by bacillariophytes with six, euglenophyte with six and dinophyte and cryptophytic with one representative each, there was no great difference regarding the quality of the phytoplankton between the different collection points, independent of the sampling period. Cyanophyte blooms can cause undesired changes in shrimp farming environments, this class of phytoplankton in high concentrations generate toxins that affect the development of the animals and in extreme cases cause mass killings, which result in losses to producers (ZAFAR *et al.*, 2015; MORALES-COVARRUBIAS *et al.*, 2016; AJIN *et al.*, 2016). According to Maia *et al.* (2013), the most desirable phytoplankton groups for shrimp farming are chlorophytes and bacillariophytes because they contribute to the diet of zooplankton and shrimps, being an excellent nutritional source for these animals. Within the cyanophyte division, the genera that presented the highest densities in WPE, wPE and in the supply channel were *Chroococcales* sp., *Cylindrospermopsis* sp.,

*Pseudanabaenaceae* sp., *Nostocales* sp., *Geitlerinema* sp. In the chlorophyte division the genera *Chlorococcales* sp. and *Scenedesmus* sp. were noticeable, in the bacillariophyte the genera *Cyclotella* sp. and *Nitzschia* sp. were also prominent, while the genera of the other divisions did not present representative values. The genus with the highest density was *Nostocales* sp. with a mean value of 264.975 cells/mL in the supply channel, 992.085 cells/mL in WPE ponds and 828.120 cells/mL in wPE. The only genus that showed significant difference ( $p < 0.05$ ) in relation to phytoplankton density between the collection sites was *Geitlerinema* sp., which belongs to cyanophyte division, and this quantitative difference was observed between the samples of the supply channel and the samples of WPE and wPE ponds.

According to Porchas-Cornejo *et al.* (2013), copepods belonging to the Crustacea group occur with greater predominance in the zooplankton community in shrimp farming, and these organisms, which were found in abundance in the present study, are of great importance because they are rich in lipid and protein nutrients that are assimilated when predated by the cultivated shrimps (MARTÍNEZ-CÓRDOVA, 2011). The highest cell density was observed in an unidentified species of nauplii, with absolute values above 600 org./L, the highest average density of these organisms was recorded in wPE ponds with 386 org./L and the lowest in the supply channel with 106 org./L, while for copepods higher density was also observed in wPE ponds with 259 org./L, values higher than those recommended for shrimp farming by Nunes (2001).

In the plating of the samples, no growth of typical bacterial colonies of *Vibrio* spp. occurred in TCBS culture medium, indicating a healthy growing environment. Bacteria of the genus *Vibrio* spp. are microorganisms that present a wide range of distribution, mainly in marine environments and in areas influenced by the sea, such as estuaries and coastal lagoons (KEEN *et al.*, 2012). According to Boonchuen *et al.* (2018), the presence of halophilic bacteria, which develop in high salinity environments, in shrimp cultivation is linked to microbiota from estuarine areas. Since *Vibrios* spp. are typical bacteria of marine environments, this should be the most likely cause why these microorganisms were not detected in any of the samples collected on the farm. These observations were also identified by Rocha (2016) who found the influence of salinity on the quantification of *Vibrios* spp. in marine shrimp farms.

## 5 CONCLUSIONS

There is not enough evidence to show that the differences found in water quality in *Penaeus vannamei* cultivation are due to the application of the pyroligneous extract. A plausible hypothesis for these findings should be related to the growing environment, as it generates distinct characteristics in the water quality parameters. Although the WPE treatment presents differences in relation to the wPE in some water quality parameters, no discrepancy was found in the productive performance of the cultivated shrimps.

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Table 1 Water quality of a *Penaeus vannamei* cultivation, for 99 days in 1-hectare ponds, submitted to treatment with pyroligneous extract (WPE) and without pyroligneous extract (wPE) (data are mean  $\pm$  standard deviation).

Parameters	Treatments		T-test
	WPE	wPE	<i>p</i>
Dissolved oxygen (mg/L)	8.30 $\pm$ 0.17a	6.81 $\pm$ 0.46b	0.007
Temperature (°C)	29.44 $\pm$ 0.61	29.16 $\pm$ 0.83	0.069
Ph	7.85 $\pm$ 0.37a	8.45 $\pm$ 0.23b	0.048
Salinity (ppt)	1.74 $\pm$ 0.31	1.26 $\pm$ 0.02	0.059
Total alkalinity (mg/L eq.CaCO <sub>3</sub> )	88.90 $\pm$ 2.00a	81.46 $\pm$ 4.16b	0.034
Total hardness (mg/L eq.CaCO <sub>3</sub> )	406.66 $\pm$ 51.31a	298.66 $\pm$ 8.32b	0.034
Calcium (mg/L)	50.93 $\pm$ 2.57	49.60 $\pm$ 1.60	0.25
Magnesium (mg/L)	67.20 $\pm$ 13.36a	41.90 $\pm$ 1.47b	0.041
Nitrate (mg/L)	<0.001	<0.001	-
Nitrite (mg/L)	<0.001	<0.001	-
TAN <sup>1</sup> (mg/L)	0.46 $\pm$ 0.33	<0.01	-
Phosphate (mg/L)	<0.001	<0.001	-
Silica (mg/L)	5.63 $\pm$ 3.86a	16.16 $\pm$ 3.93b	0.015
Turbidity (UNT)	90.00 $\pm$ 29.05	121.00 $\pm$ 19.16	0.11
Chlorophyll (µg/L)	196.46 $\pm$ 112.42	244.26 $\pm$ 104.97	0.302

<sup>1</sup>Total Ammoniacal Nitrogen (TAN). For each water quality variable, different lowercase letters on the same line indicate a significant difference between the means by Student's t test ( $p < 0.05$ ); absence of letters, on the same line indicates absence of statistical significance between the means ( $p > 0.05$ ). < Below the detection limit.

Table 2 Productive performance of *Penaeus vannamei*, during 99 days in 1-hectare ponds, submitted to treatment with pyroligneous extract (WPE) and without the pyroligneous extract (wPE) (data are mean  $\pm$  standard deviation).

Parameters of Performance	Experimental treatments		T-test <i>p</i>
	SEP	CEP	
Final average weight (g)	10.23 $\pm$ 0.83	10.83 $\pm$ 0.45	0.177
Survival (%)	71.51 $\pm$ 9.19	73.44 $\pm$ 6.10	0.390
DGR <sup>1</sup> (g/day)	0.103 $\pm$ 0.008	0.109 $\pm$ 0.004	0.167
WWG <sup>2</sup> (g/week)	0.72 $\pm$ 0.06	0.76 $\pm$ 0.03	0.167
SGR <sup>3</sup> (%/day)	6.30 $\pm$ 0.08	6.36 $\pm$ 0.04	0.172
Productivity (kg/ha)	2,908.3 $\pm$ 189.3	3,176.6 $\pm$ 177.1	0.073
FCR <sup>4</sup>	1.68 $\pm$ 0.18	1.64 $\pm$ 0.06	0.356

<sup>1</sup>Daily growth rate (DGR); <sup>2</sup>Weekly weight gain (WWG); <sup>3</sup>Specific growth rate (SGR); <sup>4</sup>Feed conversion ratio (FCR). For each productive performance variable, different lowercase letters in the same line indicate that there is significant difference between the means by Student's t test ( $p < 0.05$ ); absence of letters, in the same line indicates absence of statistical significance between the means ( $p > 0.05$ ).

Table 3 Average phytoplankton density of the most representative groups in a *Penaeus vannamei* cultivation. Supply channel (SC), ponds with application of the pyroligneous extract (WPE) and without the pyroligneous extract (wPE).

Groups	Average phytoplankton density (cell/mL)			ANOVA <i>p</i>
	Sample location			
	SC	WPE	wPE	
CYANOPHYTA				
<i>Chroococcales</i> sp.	20,580 ± 18,779	64,118 ± 66,125	48,553 ± 52,795	0.586
<i>Cylindrospermopsissp.</i>	64,500 ± 53,580	53,715 ± 8,684	151,234 ± 103,539	0.232
<i>Geitlerinemasp.</i>	29,156 ± 20,043b	98,352 ± 21,057a	116,556 ± 20,442a	0.004
<i>Nostocales</i> sp.	264,975 ± 229,485	992,085 ± 649,575	828,120 ± 79,766	0.144
<i>Pseudanabaenaceaes.</i>	58,100 ± 10,780	78,349 ± 59,701	155,388 ± 32,595	0.052
CHLOROPHYTA				
<i>Chlorococcales</i> sp.	4,896 ± 1,254	7,674 ± 7,003	7,838 ± 3,588	0.694
<i>Scenedesmus</i> SP.	10,977 ± 8,702	24,671 ± 25,814	12,562 ± 4,308	0.548
BACILLARIOPHYTA				
<i>Cyclotella</i> sp.	6,712 ± 3128	10,415 ± 5,827	5,322 ± 640	0.314
<i>Nitzschiasp.</i>	2,595 ± 153	3,610 ± 1,030	6,107 ± 5,583	0.451

For phytoplankton density, different lowercase letters on the same line indicate a significant difference between the means by Tukey's test ( $p < 0.05$ ); absence of letters on the same line indicates absence of statistical significance between the means ( $p > 0.05$ ).

Table 4 Average zooplankton density of the most representative groups in a *Penaeus vannamei* cultivation. Supply channel (SC), ponds with application of the pyroligneous extract (WPE) and without the pyroligneousextract (wPE).

Groups	Average zooplankton density (org./L)			ANOVA <i>p</i>
	Sample location			
	SC	WPE	wPE	
ROTIFERA				
<i>Brachionus</i> sp.	101 ± 74	30 ± 52	90 ± 65	0.408
CRUSTACEA				
<i>Copépodo</i>	245 ± 109	259 ± 269	191 ± 71	0.880
<i>Náuplio</i>	106 ± 184	386 ± 279	308 ± 144	0.313
PROTOZOA				
<i>Ciliado</i>	80 ± 36	35 ± 9	284 ± 401	0.428

For zooplankton density, different lowercase letters in the same line indicate a significant difference between the means by Tukey's test ( $p < 0.05$ ); absence of letters, in the same line indicates absence of statistical significance between the means ( $p > 0.05$ ).

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